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TECHNICAL MANUSCRIPT 564

THE GROWTH OF VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS IN HUMAN DIPLOID CELL STRAIN WI-38

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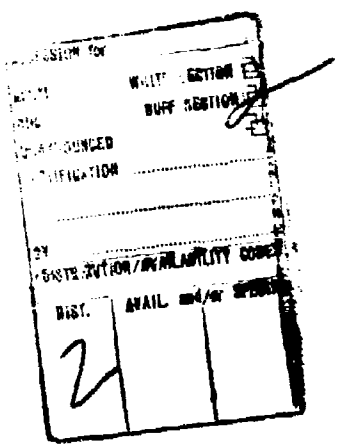

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THE GROWTH OF VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS IN HUMAN DIPLOID CELL STRAIN WI-38

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Medical Investigation Division
MEDICAL SCIENCES LABORATORIES

Project 1B662706A72

October 1969

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ABSTRACT

We demonstrated that Venezuelan equine encephalomyelitis (VEE) replicated in and adapted rapidly to human diploid cell strain WI-38. Peak titers of as high as $10^{9.9}$ $MICLD_{50}$/ml were obtained after a 48-hour incubation period at low passage levels in Eagle basal medium supplemented with 3% calf serum. VEE virus replicated poorly in serum-free medium. Propagation of the virus was accompanied by the production of hemagglutinin and cytopathogenic effect. These studies suggest that the WI-38 strain might be an appropriate source for the production of VEE virus for vaccines.

I. INTRODUCTION*

Venezuelan equine encephalomyelitis (VEE) virus is a member of the group A arboviruses. This virus produces disease in equines and is known to infect man.[1-3] VEE virus grows in Maitland-type chick embryo tissue,[4] in tissue cultures of Chang's human liver and conjunctiva, monkey kidney, chicken embryo, embryo mouse lung, guinea pig kidney, and L cells,[5] and in HeLa cells.[6]

This report describes the growth of VEE in human diploid cell strain WI-38, a cell strain derived from the lungs of a human embryo. This cell strain has a limited life span of about 50 generations and is free of known contaminating viruses.[7] Diploid cell strains have supported the replication of human and animal viruses.[7-9] It was of interest to us to investigate the ability of WI-38 cells to support the growth of VEE virus as a possible method for production of virus for preparation of inactivated vaccines.

II. MATERIALS AND METHODS

A. VIRUS STRAIN

The Trinidad donkey brain strain of VEE virus[5] was acquired at this laboratory as a 10% chick embryo suspension in beef heart infusion broth (BHIB). Seed virus E was prepared from the 14th egg passage in 10-day embryonated eggs. The seed virus was a 15% embryo suspension in BHIB and contained $10^{10.9}$ mouse intracerebral 50% lethal doses ($MICLD_{50}$)/ml.

B. VIRUS TITRATIONS

Groups of five Swiss albino mice obtained from the Fort Detrick Animal Farm were inoculated intraperitoneally (IP) with 0.2 ml and intracerebrally (IC) with 0.03 ml of serial 10-fold dilutions of virus in BHIB. The ID_{50} end points were calculated by the method of Reed and Muench.[10]

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C. CELL CULTURES

Human diploid cell strain WI-38,[11] obtained through the courtesy of Dr. Norman Swack of these laboratories and from Mr. George Gardiner of N.I.H., was grown as monolayers in glass or plastic flasks. Monolayers also were obtained from Flow Laboratories, Rockville, Maryland. Cell cultures were fed Eagle basal medium (BME)[12] with Hanks salts* (HBME), supplemented with 10% fetal calf serum (FCS). Antibiotics were added to the medium to give final concentrations of either 100 units of penicillin and 100 μg of streptomycin or 50 μg of aureomycin per ml.

D. HEMAGGLUTINATION TEST

Culture fluids were tested for the presence of hemagglutinin (HA) as described in a previous publication[13] by the microtiter technique[14] using goose erythrocytes.

E. INOCULATION OF CELL CULTURES

Monolayers were washed and inoculated with various dilutions of VEE virus as described below. After adsorption for 45 minutes at 35 C, the inoculum was removed and the cell sheet was washed in accordance with the experimental design. The cultures then were fed BME with Hanks or Earle salts plus 3% FCS and incubated at 35 C for designated periods of time. The infected culture fluid was harvested, clarified by low-speed centrifugation (700 x g) at 4 C, dispensed into serum bottles, and stored in a mechanical freezer at -70 C until assayed.

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* Grand Island Biochemicals Company, Grand Island, N.Y.

III. RESULTS

Preliminary attempts to propagate VEE virus in WI-38 cells revealed the presence of a toxic factor for diploid cells in the virus egg seed. The toxic factor was eliminated by diluting the seed out to 10^{-6}. Table 1 shows that when monolayers were inoculated with a multiplicity of 0.002 mouse intraperitoneal LD_{50} ($MIPLD_{50}$) of VEE virus per cell, very little demonstrable virus was present at 18 hours, the titer rose to a peak of $10^{9.0}$ $MIPLD_{50}$/ml at 42 hours, and then it decreased to $10^{6.6}$ $MIPLD_{50}$/ml at 96 hours. A 1+ cytopathogenic effect (CPE) was present at 18 hours and increased to complete destruction of the monolayer at 96 hours. HA was present in small amounts at 42 and 72 hours, but was not demonstrable at 18 and 96 hours.

TABLE 1. PROPAGATION OF VEE VIRUS IN WI-38 MONOLAYERS[a]

Hours	CPE	HA[b]	PFU/ml[c]	Log $MIPLD_{50}$/ml
18	1+	<10	1×10^2	2.0
42	2+	40	5.1×10^7	9.0
72	3+	40	3.5×10^7	8.1
96	4+	<10	2.7×10^6	6.6

a. Cell sheets were washed 3 times with BHME and inoculated with a multiplicity of 0.002 $MIPLD_{50}$ of virus, and the inoculum was removed after an adsorption period of 45 minutes. Maintenance medium was HBME plus CS.
b. Reciprocal of highest dilution causing complete agglutination pattern of goose red cells with 0.05 ml.
c. Performed by Dr. E. Zebovitz.

A. EFFECT OF CALF SERUM ON VIRUS YIELD

Monolayers were washed three times with saline A to remove residual serum and then inoculated with serial 10-fold dilutions of VEE virus in phosphate buffered saline (PBS). The virus had previously undergone one passage in WI-38 cells. After a 45-minute adsorption period at 35 C, the nonadsorbed virus was removed and the cell sheets were washed twice with BME with Earle salts (EBME). The infected monolayers then were fed maintenance medium consisting of EBME with or without 3% calf serum. Samples of the culture fluid were taken at this time and at 24-hour intervals. Figure 1 shows the virus titers obtained after incubation at

35 C. The results indicate that calf serum is required for propagation of VEE in WI-38 cells. In the presence of serum, titer rose sharply at 24 hours, with a peak at 48 hours followed by a gradual decrease in titer through 120 hours of incubation. Calf serum also appears to protect the virus on the cell sheet, since in the absence of serum no virus could be detected by mouse inoculation at zero time. At this time, virus titers were as high as 4.2 $MIPLD_{50}$/ml in the culture fluids supplemented with serum. Titers in the culture fluids of monolayers maintained on serum-free medium did not exceed 5.1 $MIPLD_{50}$/ml. Identical peak titers ($10^{9.1}$ $MIPLD_{50}$/ml) were obtained in serum containing medium inoculated with 10^{-1} or 10^{-2} dilutions of virus. However, the titer in the flask inoculated with a 10^{-3} dilution was 0.7 log lower.

B. PASSAGE OF VEE VIRUS IN WI-38

Monolayers grown in 32-oz prescription bottles were washed three times with HBME and inoculated with 2 ml of virus seed E, diluted 10^{-6} in HBME. After a 45-minute absorption period at 35 C, the inoculum was removed and the cell sheet was washed twice with HBME. The infected monolayers were fed 80 ml of HBME supplemented with 3% calf serum and incubated at 35 C for 48 hours. Culture fluids were harvested, clarified by centrifugation at 700 x g, and stored at -70 C until passaged or assayed. Undiluted infected culture fluids were used as inocula for further passage.

The data indicate that acaptation of VEE virus takes place readily (Table 2). In one experiment (lot 49), infectivity values decreased in passages one through three and then increased in the subsequent passage. The reason for the low $MICLD_{50}$ value in passage 3 could not be ascertained. In the second experiment (lot 50), higher titers were attained after an initial drop on the first passage. An $MICLD_{50}$ value approximately as high as that contained in the original seed virus was obtained in passage 3, lot 50, and in passages 2a and 3, lot 59. Approximately 9 logs of virus/ml were obtained on initial passage of VEE virus with lots 58 and 59.

HA titers ranged from <1:2 to 1:128 per 0.05 ml. Passage of VEE virus in WI-38 does not appear to enhance the production of HA since an increase in titer was obtained only in two instances (lot 50 and 58) and this occurred only in the second passage.

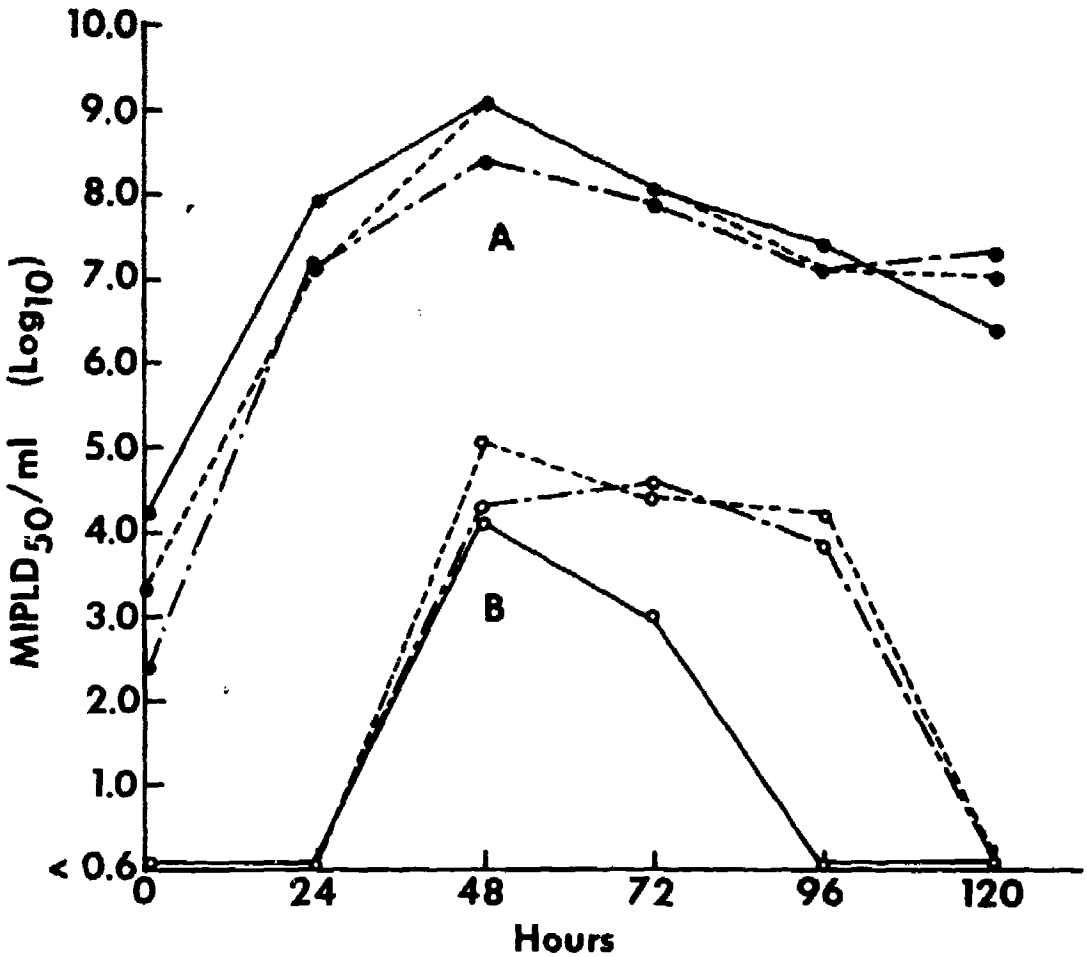

FIGURE 1. Effect of Inoculum Concentration and Serum on Growth of VEE Virus in WI-38 Cells. A, EBME medium supplemented with 3% calf serum; B, serum-free medium. Symbols: ——, 10^{-1}; ----, 10^{-2}; — · — · , 10^{-3} dilutions of inoculum.

TABLE 2. PASSAGE OF VEE VIRUS IN WI-38 MONOLAYERS

Lot No.	Passage No.	Reciprocal HA Titer/0.05 ml	Log $MIPLD_{50}$/ml	Log $MICLD_{50}$/ml
Seed E	0		10.0	10.2
49	1	2	7.9	8.7
	2	4	7.5	8.7
	3	2	7.7	<6.0
	4	2	8.2	8.1
	4a	4	8.3	ND[a]
	5	2	7.5	8.3
50	1	4	8.3	8.1
	2	32	9.1	ND
	2	64	9.2	9.1
	3	4	8.7	9.9
	3	4	8.2	ND
	4	<2	7.9	7.8
	4a	ND	8.2	9.7
	5	ND	8.9	9.2
58	1	8	8.7	9.1
	2	32	8.9	9.1
	3	<2	8.5	8.5
59	1	64	9.1	9.0
	2	32	8.9	9.1
	2a	128	9.1	9.7
	3	64	9.2	9.8

a. ND = not done.

IV. DISCUSSION

Human diploid cell strain WI-38 appears to be an excellent substrate for the propagation of VEE virus. Titers as high as $10^{9.9}$ $MICLD_{50}/ml$ were obtained at low passage levels.

The data indicate that the yield of VEE virus is affected by the presence of calf serum in the maintenance medium. Thus, there was little virus yield in monolayers maintained in serum-free medium, but high yields were obtained with the addition of serum. Similar results have been reported by Plotkin, Boué, and Boué[15] with rubella virus in WI-38 cells. Low yields obtained in serum-free cultures may be due to instability of VEE virus. Hardy and Brown[16] reported that VEE virus was less stable in medium 199 at 37 C than in the same medium without serum.

Infection of monolayers with the range of multiplicities used here had little effect on the virus yields. Approximately the same peak titers were obtained at 48 hours of incubation in serum-containing medium in cell cultures inoculated with either 10^{-1}, 10^{-2}, or 10^{-3} dilutions of tissue culture virus seed. The greatest difference in peak titers observed was 0.7 log and was not considered to be significant.

VEE virus appears to propagate more slowly in WI-38 cells than in L or chick fibroblast cells. Peak titers were observed at 48 hours of incubation in WI-38 cells but have been reported to appear 24 hours earlier in L cells[16] and in chick fibroblast cells.[17] It is of interest to note that although peak titers appeared after 48 hours of incubation in WI-38, maximum CPE occurred 48 hours later. Hardy and Brown[16] found that CPE appeared 12 to 24 hours after peak titers were reached in L cell monolayers.

Passage of VEE virus through WI-38 did not increase the quantity of HA, and in some experiments the ability to produce this antigen was lost after a few successive passages, although the infective titer for mice remained at a high level. Similar observations were reported by Yershov and Vagzhanova[17] in chick fibroblast monolayers.

Since the antigenic capacity of inactivated virus vaccines is directly related to antigenic mass,[18] the production of high titers of VEE virus in human diploid cell strain WI-38 suggests that this cell strain might be an appropriate source for the preparation of an inactivated vaccine.

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13. ABSTRACT

We demonstrated that Venezuelan equine encephalomyelitis (VEE) replicated in and adapted rapidly to human diploid cell strain WI-38. Peak titers of as high as $10^{9.9}$ $MICLD_{50}$/ml were obtained after a 48-hour incubation period at low passage levels in Eagle basal medium supplemented with 3% calf serum. VEE virus replicated poorly in serum-free medium. Propagation of the virus was accompanied by the production of hemagglutinin and cytopathogenic effect. These studies suggest that the WI-38 strain might be an appropriate source for the production of VEE virus for vaccines.

14. Key Words

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Human diploid cell strain WI-38
Vaccines

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